Remarks

Reconsideration and withdrawal of the rejections of the claims, in view of the amendments and remarks herein, is respectfully requested. Claims 1, 3-6, 9, 11-12, 15, 18, 20-21, 24-37, 42-43, 45, 47, 60, 67, 69-71, 74, and 76-80 are amended, claims 81-82 are added, and claims 2, 14, 61-63, 68, 72-73, 75, and 79 are canceled without prejudice. The amendments are intended to advance the application and are not intended to concede to the correctness of the Examiner's position or to prejudice the claims prior to amendment, which claims are present in a continuation of the above-referenced application. Claims 1, 3-6, 9, 11-12, 15, 18, 20-21, 24-39, 41-45, 47, 60, 64, 67, 69-71, 74, 76-78, and 80-82 are pending.

The Examiner is thanked for the courtesies extended to Applicant's Representative in the telephonic interview conducted on July 28, 2004, in which the rejections in the previous Office Action were discussed.

The Examiner objected to claims 64, 75 and 79 under 37 C.F.R. § 1.142(b) as being drawn to a nonelected species. Claims 63 and 75 are canceled, thereby obviating the objection as it relates to those claims. With respect to whether claim 79, which depends on claim 78, is directed to a nonelected species, the Examiner is requested to consider that claim 79 is directed to a sequence for a parent nucleic acid molecule, not a synthetic sequence corresponding to the elected species, i.e., SEQ ID NO:9. Therefore, Applicant respectfully requests that claim 79 be examined with the elected group of claims and not withdrawn from consideration for being directed to subject matter in a nonelected group.

At page 3 of the Office Action, the Examiner objected to claims 1, 47, 63, 67-68, and 73 for the recitation of "and" after "transcription factor binding sequences." The amendment to delete the respective "and" obviates the objection.

The 35 U.S.C. § 112, Second Paragraph, Rejections

The Examiner rejected claims 1-6, 9, 11-12, 14-15, 18, 20-21, 24-39, 41-45, 47, 60-63, and 67-73 under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention. This rejection is respectfully traversed.

In particular, the Examiner asserts that the following phrases are vague and indefinite: "a reduced number of transcription factor binding sequences," and/or "a reduced number of intron splice sites, poly(A) addition sites and promoter sequences," as without knowing all of the possible sequences which are considered to be transcription factor binding sequences, intron splice sites, poly(A) addition sites and promoter sequences, the number of such sequences could not be calculated. The Examiner acknowledges that "transcription factor binding sequences," "intron splice sites," "poly(A) addition sites" and "promoter sequences" are conventionally used in the art and that there are clearly art defined sequences within each category (pages 4-5 of the Office Action). Nevertheless, the Examiner asserts that as new members of each category are recognized, the scope of the claims would change.

However, the Examiner is requested to consider that even if new members of any particular class of regulatory sites are recognized, regardless of how many members are in that class, the synthetic nucleic acid molecule of the invention is one having fewer sites which, in the absence of codon selection, would otherwise be introduced.

Therefore, withdrawal of the § 112(2) rejection is respectfully requested.

The 35 U.S.C. § 112, First Paragraph, Rejections

The Examiner rejected claim 63 under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time of the application was filed, had possession of the claimed invention (a "written description" rejection). The cancellation of claim 63 without prejudice obviates this rejection.

The Examiner also rejected claims 1-6, 9, 11-12, 14-15, 20-21, 24-33, 35-39, 41-45, 47, 60, 61, 63, 67-70, 72-74, and 77-78 under 35 U.S.C. § 112, first paragraph, because the specification, while being enabling for a variant of a parent DNA molecule encoding a polypeptide identical to a polypeptide encoded by the parent DNA, having more than 25% of the codons altered and having a reduced number of transcription regulatory sequences than the parent nucleic acid or to any nucleic acid which will hybridize to SEQ ID NO:9 under high stringency conditions and encode a polypeptide having luciferase activity, does not reasonably provide enablement for any variant DNA molecule encoding a reporter polypeptide having at

least 85% identity to a wild-type reporter polypeptide, having more than 25% of the codons altered and having a reduced number of transcription regulatory sequences than the parent nucleic acid or to any nucleic acid which will hybridize to SEQ ID NO:9 under medium stringency conditions, encode a polypeptide having at least 85% identity to the polypeptide encoded by SEQ ID NO:9, have more than 25% of the codons altered and have a reduced number of transcription regulatory sequences. This rejection, as it may be maintained with respect to the pending claims, is respectfully traversed.

The specification discloses that a *Renilla* luciferase gene and a yellow-green click beetle luciferase gene (YG#81-6G01) were modified by replacing codons and reducing the number of transcriptional regulatory sequences (Examples 1-3). Codons were generally substituted with mammalian high-usage codons and not with mammalian low-usage or *E. coli* low-usage codons, so that in most cases the substituted codons did not add transcription regulatory sequences (page 53, lines 8-10; page 66, lines 24-26). Two synthetic *Renilla* luciferase sequences (Rluc ver 2 and Rluc final) and 14 synthetic click beetle luciferase sequences (GRver2, GRver3, GRver4, GRver5, GR6, GRver5.1, RDver2, RDver3, RDver4, RDver5, RD7, RDver5.1, RDver5.2 and RD156-1H9) are disclosed.

In particular, the Examiner is requested to reconsider that one amino acid substitution is present in the protein encoded by the two synthetic *Renilla* luciferase sequences relative to a wild type *Renilla* luciferase sequence, and a number of amino acid substitutions were introduced to codons in synthetic click beetle luciferase genes relative to a parent luciferase sequence: GRver2-GRver5, and GRver5.1 have 1 amino acid substitution (related to a substitution associated with green light) relative to parent sequence YG#81-6G01; RDver2-RDver5 and RDver5.1 have 4 amino acid substitutions (related to substitutions associated with red light) relative to YG#81-6G01; RDver5.2 has 5 amino acid substitutions (related to substitutions associated with red light and improved spectral properties) relative to YG#81-6G01; and RD156-1H9 has 9 amino acid substitutions (related to substitutions associated with red light, improved spectral properties and improved luminescence intensity) relative to YG#81-6G01 (see Figure 3 for a comparison of the amino acid sequences encoded by the synthetic click beetle luciferase sequences).

Further, numerous substitutions have been introduced into beetle luciferases without affecting the reporter property of the substitution variants (see, e.g., Kajiyama et al., Protein Engineering, 4:691 (1991)), Wood et al., J. Biolumin., 4:31 (1989), Wood et al., J. Biolumin., 5:107 (1990) and Sala-Newby et al., Biochem. J., 279:727 (1991)), U.S. Patent Nos. 5,670,356, 6,552,179, 6,387,675 and 6,602,677 (all of record). For instance, in U.S. Patent No. 6,602,677, five mutant luciferases are disclosed that have 12, 21, 32, 37 and 37 substitutions, respectively, relative to a parent luciferase. Likewise, numerous substitutions have been introduced into other reporter proteins, such as GFP (see U.S. Patent No. 5,874,304, a reference cited against the claims under 35 U.S.C. § 103(a)), chloramphenicol acetyl transferase (see the enclosed abstract for Murray et al., J. Mol. Biol., 254:993 (1995)) and β-glucuronidase (see the enclosed abstract for Matsumura et al., Nat. Biotechnol., 17:696 (1999)). Thus, it is well within the skill of the art worker to predictably substitute amino acids in a reporter protein, e.g., substitute up to 10% or more of the residues.

Hence, Applicant's specification fully satisfies the requirements of 35 U.S.C. § 112(1).

The 35 U.S.C. § 103(a) Rejection

The Examiner rejected claims 1-6, 9, 11-12, 14-15, 20-21, 24-39, 41-45, 60-63, 67-70, 72-74, and 76-77 under 35 U.S.C. § 103(a) as being unpatentable over Sherf et al. (U.S. Patent No. 5,670,356) in view of Zolotukhin et al. (U.S. Patent No. 5,874,304), Donnelly et al. (WO 97/47358), Iannacone et al. (Plant Mol. Biol., 34:485 (1997) and Pan et al. (Nucl. Acids Res., 27:1094 (1999)). As this rejection may be maintained with respect to the pending claims, it is respectfully traversed.

Sherf et al. disclose a synthetic firefly luciferase gene (*luc*$^+$) in which 3 internal palindromic sequences, 5 restriction endonuclease sites, 4 glycosylation sites, and 6 transcription factor binding sites that were present in the unmodified sequence were removed, and codons were altered at sequences specified in Table 2 to codons preferred ("more common") in mammalian cells, relative to a wild type firefly luciferase gene (*luc*). Of the twenty 6 to 30 bp regions which were modified, 6 regions included modifications with a dual purpose, i.e., one region was modified to eliminate a glycosylation site and a transcription factor binding site that was present in the unmodified sequence, three regions were modified to eliminate a transcription

factor binding site that was present in the unmodified sequence and improve codon usage, one region was modified to eliminate two transcription factor binding sites (but not improve codon usage) that were present in the unmodified sequence, and another region was modified to improve codon usage and eliminate a restriction endonuclease recognition site.

Sherf et al. also disclose that a vector encoding Luc^+ or Luc was introduced to four mammalian cell lines. NIH3T3 and HeLa cells transfected with *luc*$^+$ DNA had significantly higher levels of luciferase activity relative to NIH3T3 and HeLa cells transfected with *luc* DNA (Table 3), while CHO and CV-1 cells transfected with *luc*$^+$ or *luc* DNA had comparable luciferase activity. However, it is unclear what alterations in *luc*$^+$ DNA increased luciferase activity in mammalian cells, and why those alterations did not uniformly increase luciferase activity in all the tested mammalian cells. In contrast, a synthetic *Renilla* luciferase gene of the invention was expressed at significantly higher levels relative to a wild type *Renilla* luciferase gene in NIH3T3, HeLa, CHO and CV-1 cells (Table 10).

A humanized version of a green fluorescent protein (GFP) gene is disclosed in Zolotukhin et al. in which 88/238 of the codons in the gene were altered (column 13, lines 1-4).

WO 97/47358 discloses the preparation of synthetic hepatitis C virus (HCV) genes. In particular, it is disclosed that codons in the corresponding wild-type gene that are not the most commonly employed in humans, are replaced with an optimal codon. If a CG is created by that codon replacement, i.e., the third nucleotide in the replaced codon is C and the first nucleotide in the adjacent codon is G, WO 97/47358 discloses that a different codon is selected based on Table 5 in Lathe et al. (J. Mol. Biol., 183:1 (1985)) (page 17). Once all codon replacements are made, it is disclosed that the codon optimized gene is inspected for undesired sequences such as ATTTA sequences, inadvertent creation of intron splice sites, and unwanted restriction enzyme sites, which are then eliminated by substituting codons (pages 17-18). No explanation is provided in WO 97/47358 on how to substitute those codons and there is no recognition in WO 97/47358 that codon optimization may introduce transcription factor binding sequences.

In this regard, the Examiner is requested to consider the Rule 132 Declaration enclosed herewith executed by Monika Wood, a co-inventor of the pending claims, which provides evidence that random replacement of codons in a reporter gene inadvertently introduces transcriptional regulatory sites such as transcription factor binding sequences. For the analysis,

Ms. Wood employed similar search software and databases described in the above-identified application to determine the number of transcription factor binding sequences in a starting *gfp* sequence (SEQ ID NO:1) and a human codon optimized version thereof (SEQ ID NO:3) in Zolotukhin et al. The starting *gfp* sequence had approximately 133 transcription factor binding sequences and the human codon optimized *gfp* sequence had 17 new sites.

Iannacone et al. disclose synthetic *Bacillus thuringienesis* Bt43 genes (abstract) which encode an insect toxin. To prepare those genes, Iannacone et al. modified the nucleotide sequence of Bt43 in four target regions to avoid sequences which might destabilize mRNA, sequences such as ATTTA sequences, polyA sequences, splicing sites and A or T strings > 4, and to improve codon usage for plant expression (abstract and page 490). However, splicing sites were apparently not removed (Table 1). Moreover, Iannaconne et al. did not seek to eliminate transcription factor binding sequences.

Five constructs, one with the wild type Bt43 gene and four with synthetic Bt43 genes, i.e., BtE, BtF, BtH and BtI (see Figure 2 and Table 1), were introduced to eggplant or *Solanum integrifolium* cultures, and transgenic plants regenerated. Bt43-specific polyA+ RNA in the plants was detected by Northern blot analysis (Figure 4).

It is disclosed in Iannacone et al. that no Bt43-specific bands were detected in lanes for plants with the wild type Bt43 gene even after long exposures (page 491). Interestingly, plants expressing the BtE gene had higher levels of Bt43-specific polyA+ mRNA than plants expressing the BtF gene, a gene which had fewer $(A)_{>4}$ and $(T)_{>4}$ strings, one less ATTTA sequence, and more codons modified relative to the BtE gene. In fact, no full size Bt43-specific mRNA was detected in BtF transgenic plants in contrast to BtE transgenic plants (page 494). Thus, the additional modifications in BtF relative to BtE, i.e., additional codon substitutions, and a reduced number of ATTTA sequences and A or T strings > 4, reduced full length mRNA.

And although full length BtE polyA+ RNA was present in BtE transgenic plants, no Bt toxin was produced, leading the authors to conclude that a 1.2 Kb unmodified domain in the BtE gene is a major candidate for translational blockade (page 494).

The authors of Iannacone et al. conclude that the increased level of Bt43-specific mRNA in BtE and BtF transgenic plants compared to wild type Bt43 transgenic plants could be related

to the elimination of destabilizing sequences and that the AUUUA string in wild type Bt43 is a major candidate for the instability and untranslatability of Bt43 mRNA (page 494).

Pan et al. describe a synthetic gene derived from the merozoite surface protein-1 gene (*msp*-1) of *Plasmodium falciparum*. The synthetic gene was prepared by first back translating the corresponding wild type gene using random (not preferred) human codon replacement, choosing one master sequence, and then modifying the master synthetic sequence via alternate codon replacement to eliminate sequences that might be detrimental to efficient transcription and translation, i.e., endonuclease cleavage sites, prokaryotic promoters, poly(A) signals, exon-intron boundaries, prokaryotic factor-independent RNA polymerase terminators, inverted repeats, and long runs of purines (page 1095). Notably Pan et al. did not seek to eliminate transcription factor binding sequences in *msp*-1 and did not recognize that codon optimization may introduce transcription factor binding sequences.

The Examiner asserts that it would have been obvious to further modify the luciferase gene of Sherf et al. to both increase codon preference for humans, as each of Zolotukhin et al., Donnelly et al., and Pan et al. teach modifying a large percentage of the codons of a gene to be expressed in humans, and to remove potential promoter sequences, polyadenylation sites and splice sites in order to further increase its usefulness as a reporter gene in human and other mammalian cells. The Examiner also asserts that there would have been a reasonable expectation of success in view of the results in Zolotukhin et al., Pan et al. and Iannacone et al., which all show that such alterations of other genes that are to be expressed in evolutionarily highly distinct organisms from those in which they evolved, substantially improve the levels of expression in the new host. And because Zolotukhin et al. and Iannacone et al. teach increased expression of a desired gene in a host which is distinctly different than the organism from which it originated, and WO 97/47358 and Pan et al. teach combining codon modification and elimination of sequences which interfere with transcription or translation, the Examiner asserts that the art worker would be motivated to combine individual methods together to achieve greater improvements.

Nevertheless, the combination of references does not disclose or suggest Applicant's invention as each reference discloses a different way to modify the coding sequence of a different gene, i.e., viral genes, a gene from a parasite associated with malaria, an insect toxin

gene, or a reporter gene, to increase expression, i.e., Zolotukhin et al. disclose codon modification alone to codons employed more frequently in one organism generally throughout a green fluorescent protein gene, Sherf et al. disclose limited and targeted modification (modifications in 20 regions of 6 to 30 bp) of a firefly luciferase sequence to introduce or remove cloning sites, alter insect codons to mammalian codons, and to remove post-translation modification sites, secondary structure, and transcription factor binding sites, Iannacone et al. disclose targeted modification of four regions of a toxin gene to alter *Bacillus* codons to plant codons, and to remove polyA sequences, ATTTA sequences and strings of A or T > 4, WO 97/47358 describes codon replacement to more commonly employed codons combined with further codon substitution to remove CG residue in adjacent codons, and then inspection for ATTTA sequences, intron splice sites, and unwanted restriction enzyme sites, and Pan et al. disclose random human codon replacement yielding a population of synthetic sequences with codon substitutions, choosing one master synthetic sequence, and then modifying the master synthetic sequence via alternate codon replacement to eliminate sequences that might be detrimental to efficient transcription and translation, i.e., endonuclease cleavage sites, prokaryotic promoters, poly(A) signals, exon-intron boundaries, prokaryotic factor-independent RNA polymerase terminators, inverted repeats, and long runs of purines.

Moreover, none of the cited documents recognizes that codon replacement, whether to prepare a sequence with codons employed more frequently in an evolutionarily divergent organism optionally in conjunction with further substitutions to remove restriction enzyme sites, ATTTA sequences, splice sites, polyA sites, A or T strings, CG dinucleotides in adjacent codons, prokaryotic promoters, inverted repeats and prokaryotic factor-independent RNA polymerase terminators, may create additional transcription factor binding sites, and none of the cited documents removed transcription factor binding sites from a codon optimized gene.

The Examiner is requested to consider that after codon optimization in conjunction with removal of non-transcription factor binding sites in click beetle and *Renilla* luciferase nucleotide sequences, Applicant identified about 100 and about 60 transcription factor binding sequences, respectively. Further codon replacement to remove those sequences yielded synthetic click beetle and *Renilla* luciferase sequences with 50 and 20 new transcription factor binding sites, respectively, i.e., they were introduced by codon replacement (Examples 1 and 3). The vast

majority of the introduced sequences were subsequently removed to yield a synthetic nucleic acid molecule of the invention.

With regard to the alleged motivation provided by the cited documents, if altering codon composition in an open reading frame to codons preferred in a heterologous host alone increases expression in the heterologous host, then there would be no motivation for the art worker to make any other changes, e.g., those which may reduce aberrant transcription. Nor does any of the cited art point the art worker to which changes in combination, i.e., a combination of transcription factor binding sites, and intron splice sites, poly(A) sites and/or promoter sequences would be useful in that regard.

Further, one of ordinary skill in the art in possession of the cited art would have no reasonable expectation that any particular set of changes would improve activity in a gene that is to be expressed in a highly evolutionarily distinct cell. For example, codon alterations and a reduction in polyA sequences, strings of A or T > 4 and ATTTA sequences in a Bt43 sequence yielded mRNA but did not yield a detectable protein (BtE in Iannacone et al.), and further codon alterations and a further reduction in strings of A or T > 4 and ATTTA sequences did not yield detectable full length mRNA (BtF in Iannacone et al.). Thus, an increase in codon substitutions and a decrease in RNA destabilization sequences in a synthetic gene do not necessarily improve the transcriptional characteristics of the synthetic gene relative to the reference gene. In addition, it is unclear what changes to the Bt43 (Iannacone et al.), HCV genes (WO 97/47358), *msp*-1 gene (Pan et al.) or *luc* (Sherf et al.) sequence result in improved activity in a heterologous host and why replacement of codons in *luc* with codons preferred in mammals and other alterations which resulted in luc^+ did not improve luciferase activity in all mammalian cells which expressed Luc^+.

Accordingly, withdrawal of the § 103 rejection is respectfully requested.

CONCLUSION

Applicant respectfully submits that the claims are in condition for allowance and notification to that effect is earnestly requested. The Examiner is invited to telephone Applicant's attorney (612) 373-6959 to facilitate prosecution of this application.

If necessary, please charge any additional fees or credit overpayment to Deposit Account No. 19-0743.

Respectfully submitted,

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Date December 13, 2004

By _____
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CERTIFICATE UNDER 37 CFR 1.8: The undersigned hereby certifies that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail, in an envelope addressed to: Mail Stop AF, Commissioner of Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on this 13th day of December, 2004.

Dawn M. Poole
Name

Dawn M. Poole
Signature